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Case Report—

Myxosarcomas Associated with Avian Leukosis Virus Subgroup A Infection in Fancy Breed Chickens

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SUMMARY. Formalin-fixed suspect tumors were submitted to the Poultry Diagnostic and Research Center at the University of Georgia (Athens, GA) for diagnosis. Samples were from fancy breed chickens with a history of increased tumor prevalence in both hens and roosters. Microscopically, in all the samples, there were neoplastic proliferations of spindle-shaped cells. The matrix surrounding tumor cells stained positively with Alcian blue at pH 2.5, but neoplastic cells did not stain with periodic acid–Schiff. Immunohistochemistry stains were positive for vimentin and neuron-specific enolase and negative for desmin, smooth muscle actin, and S-100 protein. Tumors were determined to be myxosarcomas. All samples were positive for PCR targeting the gp85 avian leukosis virus (ALV) envelope protein. However, analysis of the predicted amino acid sequences in the envelope gene from three separate samples showed high similarity between them and to ALV subgroup A.

RESUMEN. *Reporte de Caso*—Mixosarcomas asociados con el virus de la leucosis aviar subgrupo A en pollos de raza de ornato. Se enviaron tumores sospechosos fijados en formalina al Centro de Diagnóstico e Investigación en Avicultura en la Universidad de Georgia (Athens, Georgia) para su diagnóstico. Las muestras provenían de pollos de ornato con una historia de prevalencia de tumores en gallinas y gallos. Microscópicamente, en todas las muestras se observó proliferación neoplásica con células fusiformes. La matriz que rodea a las células tumorales se tiñó de manera positiva con la tinción de azul alcián con un pH de 2.5, pero las células neoplásicas no se tiñeron con ácido peryódico de Schiff. Las tinciones de inmunohistoquímica fueron positivas para la vimentina y la enolasa específica de neuronas y fueron negativas para la desmina, para la actina de músculo liso y para la proteína S-100. Se determinó que los tumores eran mixosarcomas. Todas las muestras fueron positivas para la reacción en cadena de la polimerasa (PCR) dirigida a la proteína de la envoltura gp85 del virus de la leucosis aviar. Sin embargo, el análisis de las secuencias predichas de aminoácidos del gen de la envoltura de tres muestras separadas, mostraron alta similitud entre ellos y con el virus de la leucosis aviar subgrupo A.

Key words: sarcomas, ALV-A, chickens, immunohistochemistry

Abbreviations: ALV = avian leukosis virus; ALV-A = avian leukosis virus subgroup A; DES = desmin; NSE = neuron-specific enolase; S100 = S-100 protein; SMA = smooth muscle actin; VIM = vimentin

Subgroups of avian leukosis virus (ALV) of the family *Retroviridae* cause a variety of neoplasms in chickens (5). The most common manifestation of ALV infections in all subgroups, except subgroup J, is lymphoid leukosis. Before eradication programs, subgroup A (ALV-A) has been one of the more common subtypes affecting egg-laying chickens and most commonly causes lymphoid leukosis in those birds (5). Because of extensive testing currently available to the commercial poultry industry, the prevalence of ALV-A is now low or absent, as is the prevalence of tumors caused by this subgroup. However, sporadic outbreaks have been reported (1,4,10,12,14). Various types of neoplasms can occur because of ALV infections. These include mesenchymal tumors such as fibromas and fibrosarcomas, epithelial tumors such as bile duct carcinomas and tumors of endothelial origin, often hemangiomas and hemangiosarcomas (5). Typically these neoplasms are rarely reported, but there are occasional tumors in individual birds and sporadic outbreaks in noncommercial flocks (5).

CASE REPORT

Twenty-two formalin-fixed tissue samples were submitted to the Poultry Diagnostic and Research Center Diagnostic Laboratory (University of Georgia, Athens, GA) for histologic examination. The source of tissues was a flock of mixed fancy breed chickens, selected for plumage patterns, that had a recent history of increasing frequency of undetermined tumors by the owner from 0.0008% to 0.008% per week. Both hens and roosters were affected as adults, 30 wk and 40–50 wk old, respectively. The birds were vaccinated against common poultry diseases similarly to commercial leghorns. Samples submitted to the diagnostic laboratory included heads, humeral-radial-ulnar (elbow) joints, bones, and dermis, all partially replaced by large protruding masses. The dermal coverings of some of the masses were ulcerated, and ulcers were partially covered by serocellular crusts. Representative sections were taken from the samples and routinely processed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Slides were examined by light microscopy. Additional slides were examined with the use of special histochemical stains and immunohistochemistry. Thick sections totaling 100 μm per tissue sample were cut from the paraffin-embedded blocks for DNA extraction for PCR analysis.

HISTOLOGY

Microscopic examination of all the sections, regardless of sex, revealed similar changes. Masses were composed of neoplastic

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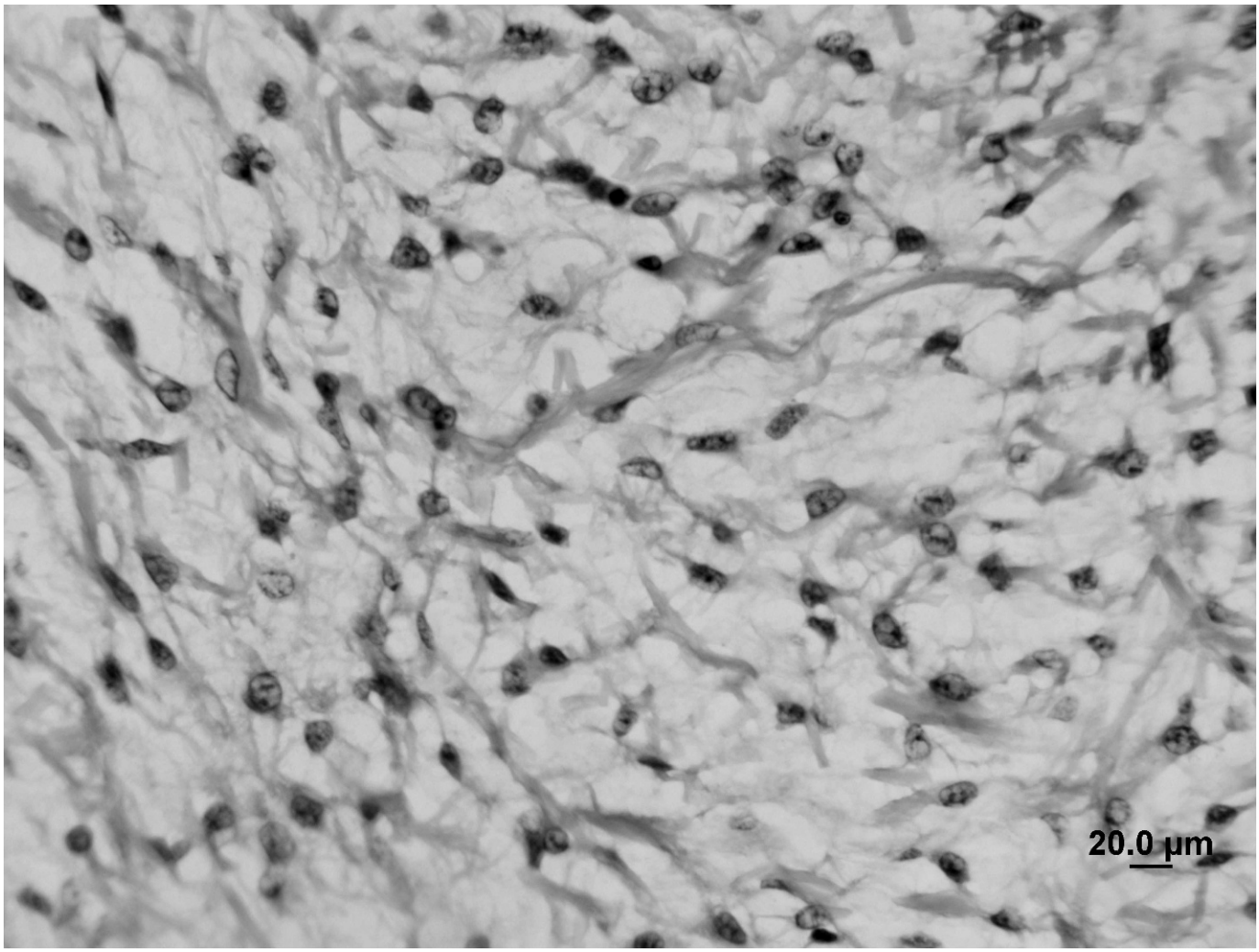

Fig. 1. Tumor, chicken. Randomly oriented spindle cells are accompanied by thin collagen fibers and surrounded by abundant matrix. H&E. Bar = 20 µm.

proliferations of randomly oriented spindle-shaped cells. These cells contained lightly basophilic cytoplasm (Fig. 1) and were separated by abundant amounts of amphophilic intercellular matrix. Tumor cells were often accompanied by thin collagen fibers. In some areas, these cells were arranged in relatively dense interwoven bundles. Spindle cells contain moderately anisokaryotic, irregularly round, ovoid to fusiform nuclei that were often vesicular. The mitotic rate was low. Neoplastic cells extensively replaced skin and bone; only fragments of bone were present in sections from elbow regions and heads. The ulcerated surfaces of some tumors were covered by serocellular crusts. Sections stained by periodic acid–Schiff (PAS) did not reveal significant staining of tumor cells or intercellular matrix. Sections stained by Alcian blue, pH 2.5, showed a pale basophilic staining of the matrix separating spindle cells.

IMMUNOHISTOCHEMISTRY

Sections of paraffin-embedded tissues 4 µm thick were placed on charged slides and immunostained with antibodies to vimentin (VIM), neuron-specific enolase (NSE), smooth muscle actin (SMA), desmin (DES), and S-100 protein (S100) according to a streptavidin-biotin-alkaline phosphatase technique (Supersensitive link label detection system; BioGenex, San Ramon, CA) following the manufacturer's recommendations. A citrate-based antigen retrieval solution (Citra Solution, BioGenex) was used on hydrated sections before immunostaining with antibodies for NSE, SMA, DES, and S100. Some sections (VIM) were alternatively treated with dilute solution of protease prior to immunostaining. All incubations were at room temperature. Monoclonal antibodies (BioGenex) to VIM, NSE, SMA, DES, and S100 were incubated on hydrated sections at room temperature for 30 min. Appropriate normal chicken tissues were used for positive and negative controls. For negative controls, the primary antibody was omitted. Samples were vimentin and NSE positive and negative for SMA, desmin, and S100. The staining was interpreted to mean that the neoplasm is a sarcoma that is not of muscle origin.

PCR ANALYSIS

DNA extraction was performed following procedures previously described (7). Amplification of the partial ALV envelope gene was attempted with the BS-up and BS-dwn primers, as described previously (13). PCR reactions were carried out with the use of a proofreading polymerase enzyme (PlatinumH Pfx DNA polymerase; Invitrogen, Carlsbad, CA) as per the manufacturer's recommendations. PCR

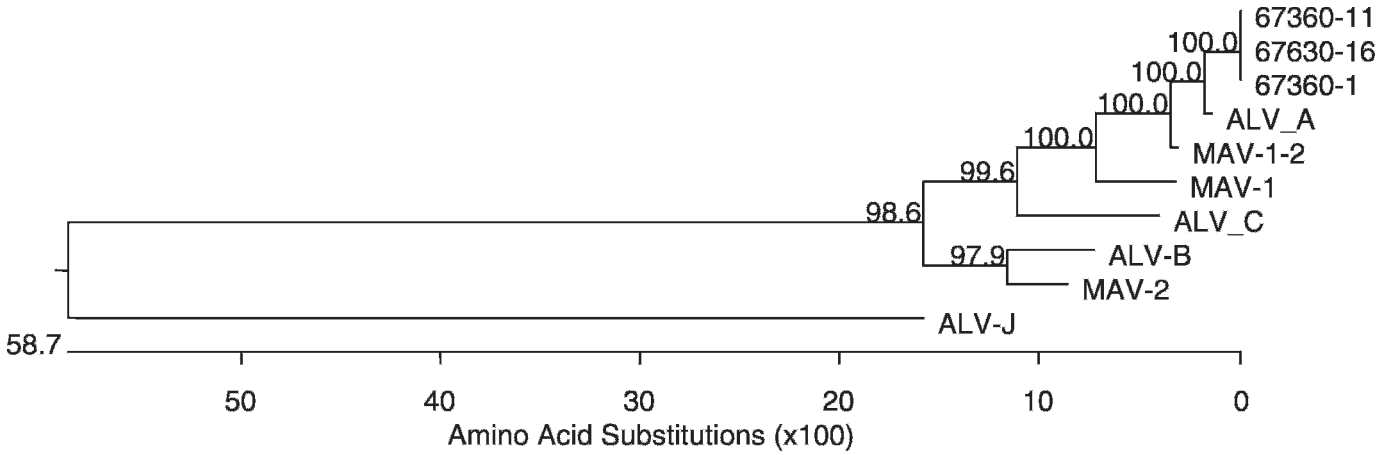

Fig. 2. Phylogenetic tree illustrating the similarities of predicted envelope gp85 (SU) proteins between the three 67360 DNA samples and representative strains of ALV subgroups: ALV-A (RCASBP(A)); ALV-B (RCASBP(B)); ALV-C (GenBank AF033808, RSV-C); ALV-D (GenBank D10652, RSV-D); ALV-J (GenBank AY027920, ADOL 7501); *ev*-1 (GenBank AY013303); MAV-1 (GenBank L10922); MAV-2 (GenBank L10923); MAV-1/2 (GenBank L10924). The amino acid sequences were aligned with the Clustal W method of the Lasergene DNAStar program. Bootstrap values of 1000 trials using the neighbor-joining methodology. Bar represents the number of changes per 100 amino acids.

products were used as templates for direct sequencing according to the dideoxynucleotide method with the BigDye v3.1 terminator kit (Applied Biosystems, Foster City, CA). Contiguous sequences were assembled with the Seqman 6.1 function in the DNASTAR sequence analysis software (DNASTAR Inc., Madison, WI). Three contiguous sequences were resolved (67360-1, 67360-11, and 67360-16) and were aligned with sequences representing subgroups A (RCASBP(A)), B (RCASBP(B)), and C (GenBank AF033808) of ALV, and also with sequences representing the myeloblastosis-associated viruses (MAVs) MAV-1 (GenBank L10922), MAV-2 (GenBank L10923), and MAV-1/2 (GenBank L10924), using the ClustalW alignment with weighted residues method in the MegAlign 6.1 sequence analysis function in DNASTAR.

Proviral DNA sequence analysis revealed ALV-A–like sequences for the 1.5-kb PCR fragment amplified from the paraffin blocks tested. All three samples shared high predicted amino acid similarity to ALV-A. The fragment amplified spanned only part of the envelope gene. However, it included the entire surface glycoprotein (SU or gp85) region of the envelope protein, which includes variable and hypervariable regions that are critical for ALV subgrouping. A phylogenetic tree of the predicted amino acid sequences of the gp85 region in the clinical samples and other reference ALVs is shown in Fig. 2. Bootstrap values were based on 1000 trials with the neighbor-joining methodology. Attempts to amplify the complete envelope gene along with the long terminal repeat region in a single reaction failed.

DISCUSSION

This case presents an unusual tumor type for avian leukosis virus infection. Interestingly, in all the samples, regardless of the sex or age of the host, there were similar neoplastic proliferations of mesenchymal cells. The immunohistochemical staining of these cells indicated that the neoplasms are sarcomas that do not exhibit any differentiation toward smooth or skeletal muscle. Although the Alcian blue staining of the matrix between the spindle cells suggests the presence of mucopolysaccharides, spindle cells did not contain the PAS+ intracytoplasmic granules that are seen in some myxosarcomas (10). Immunohistochemistry supports the histologic diagnosis of a myxosarcoma.

Detection of ALV-A from the paraffin-embedded tissues provided an etiology for the neoplasms in this group of mature chickens. There has been a previous report of ALV-A causing myxomas and neurofibromas in young laying hens (10). In that report, the tumor cell matrix stained with Alcian blue, pH 2.5, with PAS+ cytoplasmic granules. Viral matrix inclusions also were present in the cardiac myocytes of those hens. PCR and sequencing results and analysis demonstrated that the ALV detected in the tumors of those birds belonged to subgroup A and also had significant sequence homology with a MAV-1–like strain previously reported in Canadian hens (10). Ultrastructural examinations of those tumors demonstrated type C retrovirus particles. In the present case, we were able to sequence a PCR product spanning all variable and hypervariable regions in the envelope gene of ALV-A. However, outside the SU region of the envelope gene, we were not able to amplify sequences of significant length from the ALV genome involved. The inability to amplify larger fragments of the proviral genomes was most likely because of the extended period of formalin fixation before the tissues were submitted to the diagnostic laboratory (6), in that many of the samples were fixed months before submission. Analyses of different ALV genome regions such as the gp37 transmembrane envelope protein and the long terminal repeat would have allowed a better characterization of the viruses present in the tumor lesions. Another report documented commercial, egg-laying white leghorn chickens with similar tumors associated with MAV-1 infection (14). The authors documented a tumor distribution of heads and elbow joints similar to that in the present report. Myxomas have also been documented in broiler chickens (2) and domestic fowl (11). In the 1940s, Olson and Bullis reported on various types of tumors caused by suspect infectious agents and described myxomas (9). At this time, the etiology had not identified. Interestingly, myxosarcomas have also been documented to be caused by Rous sarcoma virus (8), and Rous tumors were described as neoplasms composed of stellate cells forming fibrils with a pale-staining matrix interpreted to be mucin (3). The present report documented ALV-A infection in fancy breed chickens not used for egg production or meat consumption. The source of the infection has not been determined, and multiple attempts to contact the owner to screen the flock have been unsuccessful.

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